The Genetic Basis of Phenotypic Plasticity in Maternal Investment: A Quantitative Genetic Study utilising Nutritional Geometry in the Cockroach *Naupheota cinerea*

Submitted by Joshua Vernon Charles Parry, to the University of Exeter as a thesis for the degree of Masters by Research in Biological Sciences in May 2019

MbyRes Thesis
2019
Joshua Parry

Abstract

Phenotypic plasticity is fundamental in the evolutionary process, as it allows a single genotype to display different phenotypes in response to novel environments. There is a large body of research that demonstrates the ways in which phenotypic plasticity can influence evolution, such as by permitting persistence in novel environments and revealing cryptic genetic variation, which can become genetically assimilated into a population. When plastic responses within a population differ among genotypes, a genotype by environment interaction (GxE) will exist and phenotypic plasticity will have a genetic basis of variation. This genetic basis means that plastic traits are heritable and therefore selection can target both the phenotype that plasticity produces and the plastic response itself. By better understanding the genetic basis of plasticity, the scientific community can hope to further our understanding of the evolutionary process, including how plastic traits are inherited across generations.

In this thesis, I use nutritional geometry to examine the role that dietary environment plays in the phenotypic plasticity of maternal investment in the cockroach *Nauphoeta cinerea*. By creating a half-sib pedigree, I was able to use quantitative genetics to control for individual genotypes, allowing half-siblings to be reared in different nutritional environments (a split brood half-sib design) to judge whether maternal investment varies plastically in response to the nutritional environment, and whether this plasticity has a genetic basis. I used diet eaten per day, gestation period, clutch size, and offspring lipid proportion to measure maternal investment in this study. Specifically, I used two holidic (i.e. chemically defined) diets, one with high carbohydrate content and one with low carbohydrate content, to provide different nutritional environments for the female cockroaches in the experiment. It has previously been shown that many reproductive traits, including gestation period, lipid investment into offspring, and clutch size, for *N. cinerea* are maximised on high carbohydrate diets, and thus I deemed the high carbohydrate diet a ‘high’ nutritional treatment, and the low carbohydrate a ‘low’ nutritional treatment. I found evidence of phenotypic plasticity in all four of the traits

I measured, and evidence of a genetic basis for plasticity (GxE) in diet eaten per day and offspring lipid proportion, but not for gestation period or clutch size.

Overall, my thesis provides evidence for a genetic basis of phenotypic plasticity variation in two traits that are fundamentally important for the fitness of an organism, diet eaten per day and offspring lipid proportion. I discovered that maternal investment in *N. cinerea* responds plastically to the nutritional environment, in that a single genotype can display different phenotypes depending on the diet they receive. As these two traits are linked to fitness and each other, my findings provide evidence that the plastic response to environmental conditions could evolve, producing organisms better able to persist and reproduce in a range of nutritional environments. My findings also support the theory that the role of phenotypic plasticity should be discussed in the Extended Synthesis for the evolutionary process, as these plastic traits are heritable and two show evidence of a basis in the genotype of the organism. Better understanding of the role phenotypic plasticity plays in the evolutionary process could allow us to predict population responses to changing environments, such as those presented by global climate change.

Acknowledgements

I would like to thank my supervisors; John Hunt, Alastair Wilson and Andy Russell for the use of their lab facilities, their statistical analysis and general guidance.

I would like to thank my fellow students; James Rapkin, Matthew Carey and Meaghan Castledine for their assistance in the lab.

I would like to thank my family and friends; particularly Julia King, for their support throughout the degree.

Table of Contents

Figures

Tables

Authors Declaration

The work that contributes to this thesis was conducted by Joshua Parry. All of the chapters presented in this thesis were written by Joshua Parry, with comments and editing from John Hunt, Alastair Wilson and Andy Russell.

Chapter 1: General Introduction

1.1 Phenotypic Plasticity & Genotype-by-Environment Interactions

Phenotypic plasticity is the ability for a single genotype to express multiple phenotypes in response to variation in the environment (Whitman & Agrawal 2009; Fordyce 2006; Pigliucci 2001), and has been documented as ubiquitous and potentially adaptive since as early as the 1800s (Baldwin 1896; Morgan 1896). Phenotypic plasticity has been suggested, through the publication of the Extended Synthesis, to be an important factor in the evolutionary process (Pigliucci 2009). This suggestion has been expanded on by reconsidering historical and examining contemporary research (Via & Lande 1985a; West-Eberhard 2003; Pigliucci 2005; Pigliucci 2007). Since the Extended Synthesis, evidence of the mechanisms through which phenotypic plasticity contributes to the evolutionary process has been found (Wund 2012); phenotypic plasticity allows genotypes to persist in novel environments by allowing organisms to adjust their phenotypic traits within their lifespan, which can release cryptic genetic variation which was not expressed in the previous environment (Gibson & Dworkin 2004; Le Rouzic & Carlborg 2008; Schlichting 2008). This genetic variation, expressed by the phenotype, can be selected upon, leading to the genetic assimilation (the fixing of a phenotype into the genotype by selection) and phenotypic integration (the correlation of traits within a genotype by processes such as linkage) of newly adaptive traits (West-Eberhard 2003; Waddington 1952; Badyaev 2009). This process promotes adaptive radiation (the process in which a species rapidly diversifies in response to an environmental change which opens new ecological niches) (Pfennig et al. 2010) and may confer transgenerational fitness advantages (Badyaev & Oh 2008).

When we observe a population of individuals whose plastic phenotypic response varies among genotypes, we define it as a genotype-by-environment interaction (Falconer 1952; Schlichting & Pigliucci 1998; Schmalhausen 1949; Via & Lande 1985a). At an individual level, a genotype-by-environment interaction (GxE) is the mechanistic interaction of genotype and the environment throughout the development of an organism to produce its individual phenotype (Pigliucci 2005). At a population level, we can think of a GxE as the degree of non-parallelism

among reaction norms, with the gradient of the reaction norm indicating the level of plasticity of the focal trait (Pigliucci 2005). Traits that show a GxE on the population scale must, by definition, have a genetic basis and show variation within their responses. This means that the plastic response itself can be acted upon by natural selection and hence evolve (Pigliucci 2005).

By studying phenotypic plasticity, and traits which show a genetic basis for this plasticity (i.e. genotype-by-environment interactions), we can better understand the mechanisms through which organisms adjust to their environment during their lifetime, and how that adaptability can be passed on to subsequent generations (Whitman & Agrawal 2009; Pigliucci 2009; Badyaev 2009; Badyaev & Oh 2008; West-Eberhard 2003).

1.2 Nutritional Geometry

Nutritional Geometry, also known as the Geometric Framework, is a multidimensional nutritional framework in which the concentration and ratios of nutrients in a holidic (chemically defined) diet can be varied, allowing accurate measurement of intake during feeding trials (Simpson & Raubenheimer 1995; Simpson & Raubenheimer 2012). This measurement allows us to quantify the independent and interactive elements of nutrient intake on phenotypic traits, be they somatic or reproductive. This framework has become a standard technique in experimental nutrition research (Raubenheimer et al. 2014). By varying two or more components of a particular holidic diet, one can create multiple dietary treatments composed of different ratios and total amounts of the nutrients. Because we can accurately measure nutrient intake and phenotypic traits using this method, we can statistically quantify the relationship between the two using response surface methodologies, visualised by constructing nutritional landscapes (South et al. 2011).

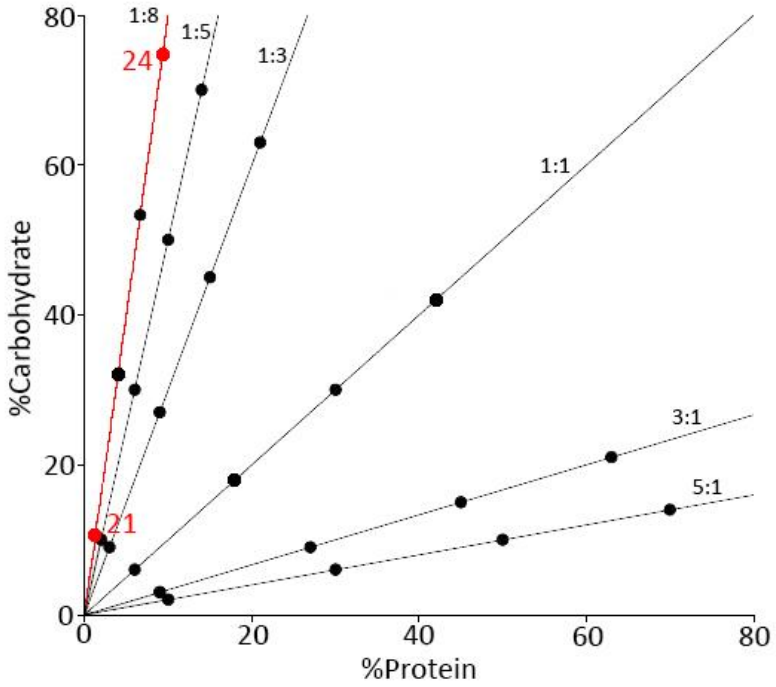

Figure 1.1 The placement of 24 diets used by South et al. (2011). Lines represent nutritional rails, dots represent specific diets used in their experiment. The diets used in this experiment (21 for 'low', 24 for 'high') are denoted by the red dots on the 1:8 rail.

Previous studies have shown that organisms tend to converge on specific nutritional rails (a vector in a multidimensional nutrient space representing the composition of a food containing a fixed proportion of nutrients and other components) (Bonduriansky et al. 2016; Simpson & Raubenheimer 1993; Raubenheimer & Jones 2006; Simpson et al. 2009). By eating, animals change their nutritional state along these nutritional rails to reach an optima that maximises or compromises certain traits (Bonduriansky et al. 2016; Simpson et al. 2009), which can be achieved by over- or under-consuming other macronutrients. Previous studies have used these convergences to determine 'high' and 'low' diets for specific organisms, such as our study species, *Nauphoeta cinerea* (South et al. 2011). South et al. (2011) and others have found that both male and female *N. cinerea* converge on a protein:carbohydrate nutritional rail of 1:4.8 (Bunning et al. 2016; Bunning et al. 2015). These high carbohydrate diets have been used in other studies as 'high' nutritional environments, and low carbohydrate diets as 'low'

environments (Bunning et al. 2015; Bunning et al. 2016; South et al. 2011; Clark et al. 1997). These ‘high’ and ‘low’ diets can be defined as those which would not and would, respectively, require compensatory feeding to achieve and optimised nutritional intake for maximised fitness (Nestel et al. 2016).

By using nutritional geometry to monitor dietary intake in my study, I should be able to accurately compare individuals on both dietary treatments, as I will have a precise measurement of their nutritional intake. I also have a wealth of studies that have previously used these diets with *N. cinerea*, giving us confidence that the two treatments can be considered ‘high’ and ‘low’ (Bunning et al. 2015; Bunning et al. 2016; South et al. 2011). By varying diet, I hoped to determine how much of the plastic response occurred due to environmental variation, and use a pedigree to establish known relatedness between individuals that would allow us to analyse the remaining variation from a genotypic and genotype-by-environment perspective.

1.3 Quantitative Genetics & the Animal Model

Quantitative genetics, also known as the genetics of complex traits, is the study of traits whose expression is the result of the action of multiple genes and non-genetic factors (Hill 2010; Lynch & Walsh 1998; Falconer 1960). The quantitative genetic framework can be used to analyse traits that do not show simple Mendelian inheritance, including those with continuously distributed phenotypes (such as size), those which take a few discrete values (such as clutch size), and binary characters that have a polygenic basis (such as survival to adulthood) (Hill 2010). Quantitative genetics is based on the statistical methods invented by Fisher (1918) and Wright (1931), is essential to our understanding of variation and covariation among relatives in a population, and allows us to measure the heritability of the complex traits in question. The fundamental importance of quantitative genetics in this study is that it allows us to create animal models, which account for the relatedness of individuals based on a pedigree, which attempt to explain the additive genetic variance of a population (Lynch & Walsh 1998; Sorensen & Gianola 2002). By applying the models to the traits concerned, I can determine whether phenotypic plasticity is present, whether the trait is influenced

by the genotype of the individual, and whether there is a genotype-by-environment interaction for the trait.

In order to make best use of the animal models offered by quantitative genetic statistical analysis, one should establish a pedigree to help control for the influence of genotype on traits, and to better explain variation within a trait (Lynch & Walsh 1998; Sorensen & Gianola 2002; Hill 2010; Pemberton 2008). Using animal models allows us to answers questions about wild populations that were previously unanswerable, such as what maintains genetic variation for phenotypic traits, and why are theoretical microevolutionary responses rarely seen in practice (Kruuk 2004). There are a vast number of studies that have managed to establish pedigrees in the wild and analyse quantitative traits using an animal model, from the methods of which are summarised by Wilson et al. (2010); Soay sheep (*Ovis aries*) from St Kilda, Scotland (Milner et al. 2000), collared flycatchers (*Ficedula albicollis*) from Gotland, Sweden (Merilä et al. 2001), bighorn sheep (*Ovis canadensis*) from Ram Mountain, Alberta, Canada (Poissant et al. 2008), great tits (*Parus major*) from Vlieland, The Netherlands (Postma & Noordwijk 2005), North American red squirrels (*Tamiasciurus hudsonicus*) from Kluane Lake, Yukon, Canada (Réale, Berteaux, et al. 2003), and many others. This version of the animal model has also been used in a laboratory setting, where establishing pedigrees is less of a challenge; black field crickets (*Teleogryllus commodus*) (Rapkin et al. 2018), sand field crickets (*Gryllus firmus*) (King et al. 2011), speckled cockroaches (*Nauphoeta cinerea*, our study species) (Schimpf et al. 2013), laboratory mice (*Mus musculus*, strain CV1) (Klingenberg & Leamy 2001), and many others. This wealth of studies shows that pedigrees can be established both in the wild and in lab populations, and provide insight into a number of fields such as microevolution, gene flow, evolutionary rates, and life-history trade-offs.

One common method of establishing a pedigree in the laboratory setting is to use a breeding design whereby individuals with assigned identities are mated and recorded, and their offspring too receive identities. By keeping track of which individuals mate with each other, it is possible to establish a ‘family tree’ of organisms of known relatedness.

1.4 *Nauphoeta Cinerea* Mating System

I have chosen to use *N. cinerea* for this experiment because there are numerous studies defining their life history, specifically reproduction (Clark et al. 1997; Harris & Moore 2005; Barrett et al. 2009; South et al. 2011; Schimpf et al. 2013). *N. cinerea* are born live in clutches of around 25 offspring. Organisms remain juvenile for around 60 days, undergoing several moultings during this period as they grow. Eclosion to adulthood is accompanied by the development of wings and after 10 days, they are sexually mature and ready to mate. Typically, mating is competitive between males through sex pheromones, the quantity and quality of which determine male dominance. Female mate choice is also determined by male sex pheromones. Gestation period for offspring is around 30 days.

Nutritionally, *N. cinerea* have maximised fitness on high carbohydrate diets at a P:C ratio of approximately 1:4.8 (Bunning et al. 2016). This maximised fitness comes in the form of increased lifespan, pheromone production, sperm number, and fertility (Barrett et al. 2009; South et al. 2011; Bunning et al. 2015). One hypothesised reason for the low protein requirements of *N. cinerea* is the presence of endosymbiotic bacteria (*Blattabacterium*) which store excess nitrogen as uric acid crystals, which can later be recycled to produce amino acids (Sabree et al. 2009; Kambhampati et al. 2013; Patiño-Navarrete et al. 2014). Unpublished findings from our lab have shown that *N. cinerea* on high carbohydrate diets produce more offspring per clutch with a higher offspring lipid proportion that survive longer under starvation (Parry et al. 2016). These factors all contributed to the choice of *N. cinerea* as our study organism.

1.5 Outline and Objectives

As previously mentioned, there are many studies that have utilised nutritional geometry and quantitative genetics separately to investigate the impact of environment on phenotypic traits. These studies have been able to reveal and explain variation in complex traits, and in some cases link that variation with the nutritional environment. There are surprisingly few studies however that use these two methods (nutritional geometry and quantitative genetics) to search for nutrition

dependent plasticity, and determine whether variation in plasticity, if present, has a genetic basis, as evidenced by variation between the plastic response among genotypes. There are examples of nutrition-dependent plasticity in complex traits such as lipid deposition into offspring, clutch size, and gestation period (King et al. 2011, Parry et al. 2016), and evidence for the evolution of plasticity (which would suggest a genetic basis) (Nussey et al. 2012), but there are no studies where the two facets of the question have been combined.

The primary objective of this thesis is to search for evidence of nutrition dependent phenotypic plasticity in complex traits (amount of diet eaten per day, gestation time, clutch size, and offspring lipid proportion i.e. allocation of resources to reproduction) using nutritional geometry, and to use a quantitative genetic approach, with a half-sibling pedigree, to determine whether this plasticity has a genetic basis of variation, as evidenced through the presence of GxEs for these traits. This research is important because it contributes to the growing body of evidence which suggests phenotypic plasticity and the evolution thereof is an important factor in the process of evolution. Understanding the relationship between phenotypic traits and the environment, including GxEs, enables us to better understand the responses of organisms to environmental change, such as that created by global climate change.

I present this thesis with a discrete research paper, which contains its own literature review, methodology, results, and discussion, in which I raise speckled cockroaches (*Nauphoeta cinerea*) of known relatedness in ‘high’ and ‘low’ nutritional environments (high and low carbohydrate, respectively), and examine plastic response in the aforementioned traits between genotypes and nutritional environments. Furthermore, I use an animal model to explain the variation in these responses, factoring in pedigree and environment, to search for GxEs for these traits.

Chapter 2: General Methods

2.1 Quantitative Genetic Approach

To determine the quantitative genetics of clutch size, diet eaten per day, gestation period and offspring lipid proportion, I used a half-sib breeding design in which male and female offspring from each full-sib family were mated and female offspring allocated onto a ‘high’ or ‘low’ diet regime after eclosion. The half-sib breeding design was established by mating 20 virgin males to 6 virgin females each (120 virgin females total) (see Figure 2.1). This mating yielded 716 female offspring from 20 paternal families who were reared in a family plastic container (17 x 12 x 6 cm) with food and water *ad libitum* (rat food (SDS Diets, Essex, UK) and plastic test tubes plugged with cotton wool (10 ml)). After eclosion, females were collected and stored in small plastic containers (11 x 11 x 3 cm) with water *ad libitum* (plastic test tubes plugged with cotton wool (10ml)) and their specified diet regime (‘high’ diet 24 or ‘low’ diet 21). Fresh diet was provided every five days until birth. Fresh water was provided every 5 days. Mating occurred at sexual maturity (circa 10 days) with virgin males from other families (kept in identical conditions save for diet (rat food *ad libitum* (SDS Diets, Essex, UK)). After mating, males were removed. Within 24 hours of the female giving birth, she and her offspring were frozen for lipid analysis.

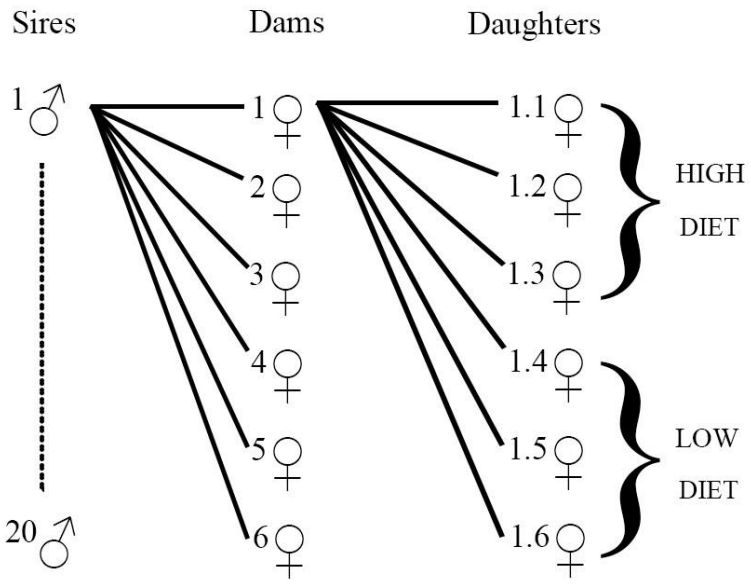

Figure 2.1 Experimental design for the split brood half-sibling breeding experiment used in this study. This design mated each of 20 fathers (sires) to 120 mothers (dams). Each dam produced a minimum of 6 female offspring, half of which were randomly allocated to the good diet at adulthood, and the remaining half to the poor diet.

2.2 Diet Manufacture according to Nutritional Geometry

The diets in this thesis needed to vary in both the combination and concentration of nutrients, in order to meet the requirements of nutritional geometry, and examine the effects of specific macronutrients on complex traits. I created 2 artificial, holidic diets with a protein:carbohydrate ratio of 1:8 that varied in absolute amount of protein and carbohydrates (the 'high' diet having 7 times more protein and carbohydrates than the 'low') and maximised female cockroach fitness, using the established protocol outlined in Simpson & Abisgold (1985), as utilised in previous *N. cinerea* studies (South et al. 2011; Bunning et al. 2015). To create a digestible, 'powdered' form of each diet, I mixed protein, consisting of casein, albumen and peptone in a 3:1:1 ratio, and digestible carbohydrates, consisting of sucrose and dextrin in a 1:1 ratio, for both diets. Both diets contained the following in equal amounts; Wesson's salts (2.5%), ascorbic acid (0.28%), cholesterol (0.55%) and vitamin mix (0.18%). The diet mixture of proteins, carbohydrates and micronutrients was diluted to the necessary amount through the addition of

crystalline cellulose which is indigestible to the majority of insects (Martin et al. 1991).

To make the vitamin mix, each component was weighed out individually, using a microspatula and microbalance, which were then mixed in a pestle and mortar. This mixture was then stored in an airtight container in a freezer at -20˚C until needed. To make the main body of each diet the required amounts of cellulose and casein were added to a large glass beaker. In a separate smaller beaker, the specified, constant amount of cholesterol was added, in addition to linoleic acid which was added to the cholesterol using a pipette afterwards. This cholesterol/linoleic acid mixture was dissolved thoroughly in chloroform and then added to the dry cellulose/casein mix. The wet diet mixture was left in a ventilated fume hood for 24 hours and stirred at regular intervals to allow the chloroform to evaporate. After a period of 24 hours, the required amounts of Wesson salt's, sucrose, dextrin, peptone, albumin and ascorbic acid were added. The specified amount of vitamin mix was then added to the small beaker and dissolved in 20% pure ethanol, before being added to the large glass beaker. Clean spatulas and weighing boats were used to weigh out each new ingredient, and diets were stirred thoroughly upon the addition of each ingredient, to prevent contamination.

The wet diet mix was then blended in a domestic kitchen food processor for approximately 2 minutes, before being dispensed into a Pyrex baking tray and placed in a drying oven at 30˚C. The diets were then blended every 24 hours until dry, upon which they were ground using centrifugal mill into a homogenous fine powder and stored in an air tight contained, in a freezer at -20˚C until needed.

2.3 Feeding Regime

Diet was given in feed dishes created by gluing an upturned plastic vial lid (1.6 cm diameter, 1.6 cm deep) in the middle of a plastic petri dish (5.5 cm diameter) and water was provided ad libitum in a 5 ml test tube plugged with cotton wool. The design of these dishes meant that females were only able to consume the diet provided and uneaten food could be collected into the dishes if spilled during feeding. Diet was weighed using an electronic balance (Ohaus Explorer Professional EP214C, Switzerland) before allocation and replaced every 5 days

with fresh weighed diet. Removed diet was dehydrated in an oven (Binder FD115, Germany) at 30°C for 72 hours before being reweighed to determine quantity eaten by each female. Prior to weighing, any dried faeces was removed with forceps. Total eaten was calculated as the difference in dry weight of diet before and after feeding. Total eaten was converted to a weight of P and C ingested by multiplying the proportion of these nutrients in the diet (South et al. 2011).

2.4 Measuring Traits

After birth female cockroaches and their clutches were frozen at -20˚C and stored until total body lipid analysis could be performed. Lipid extraction was undertaken following the methodology outlined in (South et al. 2011). Each cockroach was defrosted at room temperature and an incision made along the abdomen using dissecting scissors to allow dichloromethane:methanol (DC:M) to fully penetrate the abdominal cavity. The cockroach was then dried at 60˚C for 24 hours and weighed using an electronic balance. Each cockroach was then placed in 20ml of a 2:1 (v/v) solution of DC:M and agitated for 48 hours at 100rpm to extract lipids. Cockroaches were then removed and dried again for 24 hours at 60˚C and reweighed. The difference between weight pre- and post-extraction was taken as lipid mass.

The same protocol was followed for clutches save for the abdominal incision, which was uneccessary due to the small size of the organisms. Clutches were analysed in their family groups rather than individually and required only 10ml of DCM due to their size.

Gestation time was recorded by tallying the days between mating and birth. As each mating was supervised and recorded, and each birth was recorded, estimating gestation time to the nearest day was possible.

Cutch size was determined by placing the mother and her offspring into the freezer at -20˚C until dead. After this time, the mother was retrieved and stored separately to the clutch, and offspring could be counted manually before being stored.

Chapter 3: The Genetic Basis for Nutrition Dependent Phenotypic Plasticity in Maternal Investment in the Cockroach *Nauphoeta cinerea*: a study using Nutritional Geometry and Quantitative Genetics

3.1 Abstract

Phenotypic plasticity, the ability of a single genotype to display different phenotypes in response to novel environments, has been shown to be fundamental in the evolutionary process. The various ways in which phenotypic plasticity can influence evolution has been demonstrated in a large number of studies. When plastic responses within a population differ among genotypes, a genotype by environment interaction (GxE) will exist and phenotypic plasticity will have a genetic basis of variation, meaning that plastic traits are heritable and become the target of selection, and therefore evolve. If phenotypic plasticity does not have a basis in the genome, it cannot evolve. Relatively few studies have combined the fields of nutrition and plasticity to determine the effect of nutritional environment on plastic responses, and to determine whether these responses have a genetic basis. In this thesis, I use nutritional geometry to examine the role that dietary environment plays in the phenotypic plasticity of maternal investment in the cockroach *Nauphoeta cinerea*. I used quantitative genetics to control for individual genotypes, allowing me to determine whether a nutritionally dependent plastic response was occurring, and whether these responses showed evidence of a genetic basis. It has previously been shown that many reproductive traits, including gestation period, lipid investment into offspring, and clutch size, for *N. cinerea* are maximised on high carbohydrate diets, and thus I used these diets as my nutritional environments. I found evidence of phenotypic plasticity in all four of the traits I measured, and evidence of a genetic basis for plasticity (GxE) in diet eaten per day and offspring lipid proportion, but not for gestation period or clutch size. Thus, my work shows that there is nutritionally dependent phenotypic plasticity in a number of fitness-related life history traits in *Nauphoeta cinrea*, and that two of these traits show evidence of a genetic basis for plasticity. This genetic basis means that the plastic response to nutritional environment can be the target of

selection and evolve. Through this process, organisms could become better adapted to a changeable environment, such as one produced by climate change.

Key words: phenotypic plasticity, nutritional geometry, quantitative genetics, genotype-environment interaction, nutrition, *Nauphoeta cinerea*, fitness, pedigree

3.2 Introduction

Plasticity is the capacity of a single genotype to exhibit a range of phenotypes in response to variation in the environment (Fordyce 2006) and has been recognised as an important factor in evolution for well over a decade (Via & Lande 1985b; Pigliucci 2009). This plasticity can be behavioural, developmental, and physiological, and can significantly influence the fitness of an organism (West-Eberhard 2005; Monaghan 2008; Uller 2008). Plasticity promotes persistence in novel environments (West-Eberhard 2003), which can in turn reveal cryptic genetic variation (Gibson & Dworkin 2004; Le Rouzic & Carlborg 2008; Schlichting 2008) which may be beneficial to organism fitness in the novel environment. These newly adaptive traits can then be fixed in the genome by genetic accommodation (West-Eberhard 2003). This means genotypes which can show beneficial plasticity have increased fitness (Whitman & Agrawal 2009). In addition, as the environment both creates and selects among phenotypic variation which is directional and highly correlated to the specific environmental change, it provides us with a new way to view evolution, contributing to a grand unifying theory where environmentally induced phenotypic variation assumes a more important or even dominant role in the evolutionary theory (Whitman & Agrawal 2009; Badyaev et al. 2005; West-Eberhard 2003; Pigliucci 2007).

Quantitative genetics (QG) is the study of complex traits; those which are affected by the action of many genes and non-genetic factors, such as body size, obesity, and maternal investment (Hill 2010). These traits can take a few discrete values (in the case of litter size) or be binary characters with a polygenic basis (such as survival to adulthood). QG, based on the statistical methods invented by Fisher (1918) and Wright (1931), are essential to our understanding of variation and covariation among relatives in a population, and allow us to measure the heritability of the complex traits in question. Studies have shown that phenotypic plasticity is not a purely genetically defined phenomenon, rather an interaction between the genetics of an organism and its environment (Berven 1982; Via 1984; Via & Lande 1985b). The benefits of using QG to study phenotypic plasticity are that I am able to account for environmental variation and determine the genetic basis of complex traits such as maternal lipid investment.

One major source of plasticity is parental effects (Solemdal 1997; Mousseau & Fox 1998; Badyaev 2008). Parental effects occur whenever the phenotype and environment of the parents have a profound influence on the phenotype and fitness of offspring. In particular, maternal effects (a subset of parental effects) have been shown to greatly impact offspring fitness (Wolf & Wade 2009; 2016). A classic example of a maternal effect is the plastic allocation of resources to offspring. Female resource allocation by way of dietary intake has been shown to significantly affect offspring in a number of different species. In the Pied Flycatcher *Ficedula hypoleuca*, maternal nutrition is positively associated with offspring tarsus length (a proxy for body size) and immunoglobulin levels in the blood (Moreno et al. 2008). Maternal nutrition has also been shown to affect offspring sex ratios in the lizard *Amphibolurus muricatus*, where females on a poor-quality diet produced fewer clutches but larger eggs with a male sex ratio bias (Warner et al. 2007), which is adaptive (Trivers & Willard 1973).

The major limitations of studies manipulating maternal diet and examining resource allocation strategies are the lack of well-defined and controlled diets. Without these, we cannot know which specific nutrients are involved in the developmental process. In order to tackle this limitation, we can apply nutritional geometry. Nutritional geometry (also known as the Geometric Framework (Simpson & Raubenheimer 1993)) is a multidimensional nutritional framework in which the concentration and ratios of nutrients in a diet can be varied, allowing accurate measurement of nutritional intake during feeding trials. This permits the creation of high-resolution nutritional surfaces upon which a trait of interest can be mapped to determine the effect of, and interaction between, dietary components on the trait of interest (Simpson & Raubenheimer 2012). These frameworks allow us to determine how individuals regulate their nutritional intake to maximise their (and their offspring's) fitness (Bunning et al. 2016). Because organisms converge on specific nutritional rails (protein:carbohydrate ratios) (Bonduriansky et al. 2016), I am also able to determine 'low' and 'high' diets – those which do and don't require compensatory feeding to achieve an optimised nutritional intake for maximised fitness (Nestel et al. 2016). In addition to the use of nutritional geometry, I can utilise QG to account for genetic differences between organisms. This allows us to

determine the effect of genes, the nutritional environment, and their interaction on maternal investment.*Nauphoeta cinerea* cockroaches have been broadly studied with regard to nutritional geometry. It has been determined that both males and females have maximised fitness on high carbohydrate diets (P:C ratio of approximately 1:4.8 (Bunning et al. 2016)) which increased lifespan (Barrett et al. 2009), sex pheromone production (South et al. 2011), and sperm number and fertility (Bunning et al. 2015). This convergence on a nutritional rail for both sexes is unusual, as females of insect species typically require more P than males to maximise reproduction (Maklakov et al. 2008; Harrison et al. 2014; Jensen et al. 2015). A potential explanation for this is the presence of endosymbiotic bacteria (*Blattabacterium*) within specialised cells in the fat body which allow storage of nitrogen as uric acid crystals in times of protein abundance, and later recycle that nitrogen as amino acids when protein becomes scarce (Sabree et al. 2009; Patiño-Navarrete et al. 2014). Previous studies have also shown that mothers on high carbohydrate diets produce more offspring per clutch with larger lipid reserves that survive longer under food deprivation (Parry et al. 2016). This, in addition to *N. cinerea* having relatively short gestation periods (around 30 days), consistent clutch sizes (approximately 25 offspring per clutch), and easily mating and husbandry in the laboratory setting, allowed us to establish pedigrees from stock populations and provided a good system to examine the genetic basis of investment using nutritional geometry and QG.

In this study I aimed to determine the genetic basis of plasticity in *N. cinerea*. To achieve this, I manipulated the nutritional intake of cockroach mothers (specifically protein and carbohydrate) in order to examine maternal investment into offspring. By forcing mothers of known relatedness to consume ‘high’ and ‘low’ diets I could determine the effects of genetics, nutritional environment, and the interaction between the two on reproductive output. Our predictions were that mothers reared on ‘high’ diets would have a higher fitness in terms of reproductive success, and that nutrient intake would be affected both by the genotype, nutritional environment, and an interaction between the two, of an organism, thus showing transgenerational phenotypic plasticity for this trait.

3.3 Methods and Materials

3.3.1 Study Species

Experimental animals were taken from an established panmictic population of speckled cockroach (*Nauphoeta cinerea*) maintained in 10 large culture containers (50 x 35 x 30 cm) that are sustained in one incubator at 28°C ± 1°C, under a 14L:10D light:dark regime, cleaned weekly and provided with carboard for shelter, water *ad libitum* from two large test tubes (15 cm long, 3 cm dimeter) stoppered with cotton wool, and rat food (SDS Diets, Essex, UK). Juveniles were mixed randomly between culture containers each generation to guarantee gene flow.

3.3.2 Artificial Diets

Using the methodology established by South et al. (2011) I created two powdered, chemically defined diets (originally found in Simpson & Abisgold (1985), each with a P:C ratio of 1:8. One of the diets was classified as ‘high’ (9.33%P, 74.66%C P+C 84) and the other as ‘low’ (1.33%P, 10.66%C P+C 12). These diets were chosen from an array of 24 potential diets as it has been demonstrated that *Nauphoeta cinera* are capable of storing protein, and thus prefer high carbohydrate diets when given dietary choice (Barrett et al. 2009; South et al. 2011) and have been used in previous feeding experiments (South et al. 2011; Bunning et al. 2015; Rapkin et al. 2018). See Chapter 2 for more information.

3.3.3 Quantitative Genetic Breeding Design

To predict the quantitative genetics of clutch size, diet eaten per day, gestation period and offspring lipid proportion, I used a half-sib breeding design in which male and female offspring from each full-sib family were mated and female offspring allocated onto a ‘high’ or ‘low’ diet regime after eclosion in accordance with the outline in Chapter 2.

3.3.4 Feeding Regime

Experimental feeding followed protocols outlined by South et al. (2011) as outlined in Chapter 2.

3.3.5 Measuring Lipid Mass

Within 24 hours of birth female cockroaches and their clutches were frozen at -20˚C and stored until total body lipid analysis could be performed. Lipid mass was measured using the DC:M method outlined in Chapter 2. Lipid mass serves as one of our proxies for maternal investment into offspring.

3.3.6 Statistical Analysis

Quantitative genetic analyses were performed using animal models fitted in ASReml (version 3) (Gilmour et al. 2009). An animal model is a variety of linear mixed-effect model which includes genetic pedigree as a random effect, allowing for the additive genetic (co)variance for phenotypic traits to be estimated (Wilson et al. 2010). I examined four phenotypic traits: clutch size, diet eaten per day (Diet/Day) (including nutritional and non-nutritional components), gestation period, and offspring lipid proportion (OLP) as a measure of fat investment into offspring. Prior to analysis each trait was standardised to a mean of 0 and a standard error of 1 using a Z-transformation.

I constructed two models, one with G and one without, and comparing Log-likelihoods for each I first tested for the effect of genotype (G) on our four phenotypic traits (see Results). Given that genetics was a significant effect for all traits, I went on to examine possible fixed effects of diet using the same method. After concluding that diet was significant for each of our four phenotypic traits, I tested for a GxE interaction by running univariate models for each trait but split across diet treatment.

We also extracted estimates of additive genetic (co)variances, heritabilities (h^2), and genetic correlations (r_A) from these models (Table 3.3). Log-likelihoods for all our models are included in the tables, and example ASReml code can be found in Text 1. All statistical inference was based on likelihood-ratio tests (LRT).

3.4 Results

There was a significant effect of genotype (G) on clutch size, Diet/Day, gestation, and OLP (Table 3.1).For each trait, the fit of the univariate models was significantly

improved by the addition of the G interaction term (P<0.001), indicating that all traits are heritable (ranging from 0.225 to 0.446). This heritability reflects the average across both dietary treatments (i.e. both 'high' diet 24 and 'low' diet 21).

Table 3.1 Comparison of univariate models with and without the interaction term between G and the dietary environment for each of our four trait measures: clutch size, Diet/Day, gestation, and OLP.

Trait	h^2	LogL G	LogL NoG	X^2_1	P
clutch size	0.362 ± 0.0833	-329.314	-355.511	52.4	<0.001
Diet/Day	0.446 ± 0.0898	-241.112	-272.223	62.2	<0.001
gestation	0.225 ± 0.0671	-295.561	-310.763	30.4	<0.001
OLP	0.288 ± 0.0760	-335.265	-355.777	41.0	<0.001

I discovered that diet significantly affected all traits (i.e., all showed plasticity on average across genotypes; P<0.001) (Table 3.2). Clutch size, Diet/Day, gestation was, on average, significantly less on the 'high' diet treatment relative to the 'low' diet treatment. OLP was, on average, significantly higher on the 'high' diet treatment relative to the 'low' diet treatment, as predicted and shown in previous studies (Parry et al. 2016). These results are visualised by our comparison of Sire Means displayed in Figure 3.1 where the gradient of the reaction norms represents the coefficients of the traits in the model.

Table 3.2 Univariate models including dietary environment treatment as a fixed effect, showing a significant effect of dietary treatment on each of the four trait measures: clutch size, Diet/Day, gestation and OLP.

Trait	Coefficient	F	DF	P
clutch size	-0.366 ± 0.0679	29.1	1, 631.1	<0.001
Diet/Day	-0.739 ± 0.0594	154.8	1, 625	<0.001
Gestation	-0.640 ± 0.0658	94.7	1,646.6	<0.001
OLP	0.342 ± 0.0690	24.5	1, 637.7	<0.001

Finally, I investigated whether there were GxEs for our traits. I followed the same procedure of comparing models with and without GxEs. I discovered the fit of the models was significantly improved by the addition of this interaction term for Diet/Day and OLP. This was not the case for clutch size and gestation. (Table 3.3) These interactions are visualised in the reaction norms provided in Figure 3.1, with multiple crossing sire means indicating that different genotypes respond differently across dietary treatment, indicative of significant GxE interactions. These crossing sire means are more pronounced for clutch size and OLP than Diet/Day and gestation.

Table 3.3 Univariate models including GxE as an interaction term, showing a significant effect of GxE on both Diet/Day and OLP but not clutch size or gestation.

Trait	COV_A	R_{G12}	LogL GxE	LogL NoGxE	X^2_2	P
clutch size	0.301 ± 0.095	0.77 ± 0.177	-316.883	-318.674	3.582	0.167
Diet/Day	0.253 ± 0.092	0.517 ± 0.151	-202.419	-214.912	24.986	<0.001
gestation	0.195	1	-284.135	-284.378	0.486	0.784
OLP	0.219 ± 0.084	0.783 ± 0.259	-334.335	-337.518	6.366	0.041

Table 3.4 shows the additive and residual variances for each of our four phenotypic traits. When looking at those who are significantly affected by GxEs (Diet/Day and OLP), it is clear that the differences between dietary treatments are the result of differences in additive rather than residual variance (0.856 in ‘low’ versus 0.279 in ‘high’ for Diet/Day; 0.169 in ‘low’ versus 0.466 in ‘high’ for OLP) which is an indicator of strong GxEs. In contrast, the differences in residual variances between the two dietary treatments is relatively low (0.303 in ‘low’ versus 0.216 in ‘high’ for Diet/Day; 0.684 in ‘low’ versus 0.608 in ‘high’ for OLP). Referring back to Figure 3.1, we can see that those traits which have a significant GxE are also those whose reaction norms cross over the most. We can also see significant differences in heritability of these phenotypic traits between the two environments (0.739 in

'low' versus 0.564 in 'high' for Diet/Day; 0.198 in 'low' versus 0.434 in 'high' for OLP).

Table 3.4 Additive and residual variances for each of our four phenotypic traits.

Trait	Bad (21)			Good (24)		
	V_{A1}	V_{R1}	h^2_1	V_{A2}	V_{R2}	h^2_2
clutch size	0.303 ± 0.106	0.395 ± 0.083	0.434 ± 0.132	0.503 ± 0.160	0.721 ± 0.128	0.411 ± 0.114
Diet/Day	0.856 ± 0.220	0.303 ± 0.145	0.739 ± 0.138	0.279 ± 0.074	0.216 ± 0.052	0.564 ± 0.121
gestation	0.233 ± 0.119	0.839 ± 0.112	0.218 ± 0.105	0.163 ± 0.069	0.491 ± 0.064	0.249 ± 0.098
OLP	0.169 ± 0.094	0.684 ± 0.095	0.198 ± 0.107	0.466 ± 0.142	0.608 ± 0.112	0.434 ± 0.115

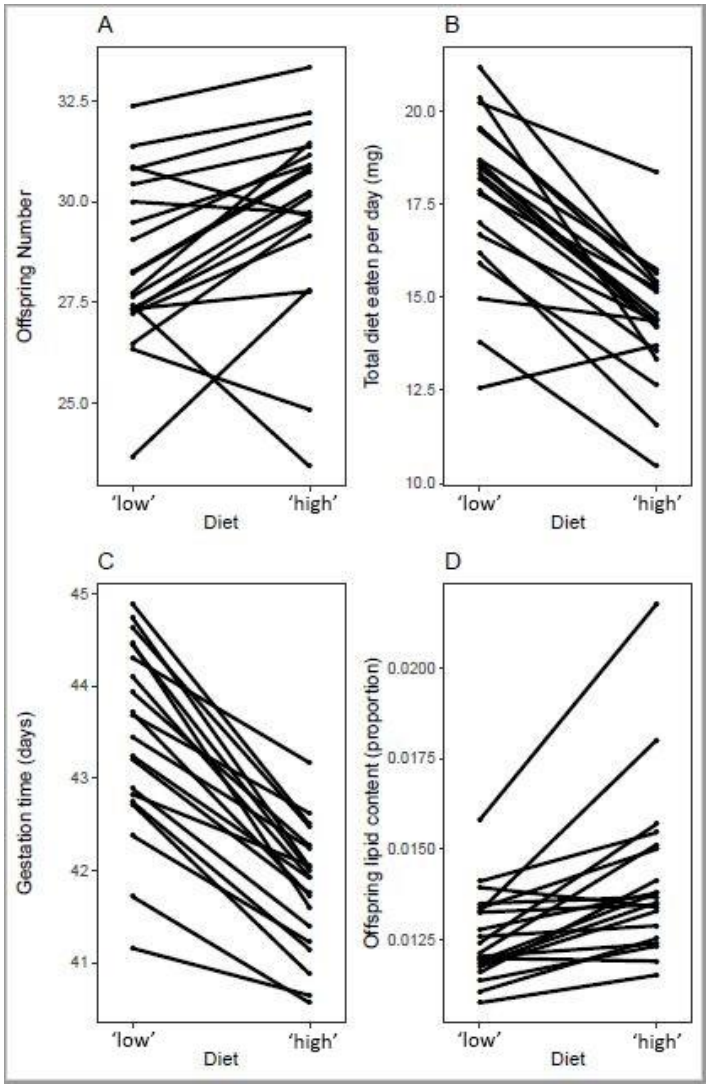

Figure 3.1 Reaction norms illustrating the genotype-by-environment interaction (GxE) for (A) clutch size (offspring number), (B) total eaten per day (mg), (C) gestation time (days), and (D) offspring lipid content (proportion, OLP) in female *N. cinerea*. In each panel, the lines represent the response of a given genotype (halfsib family) across dietary environments.

3.5 Discussion

Phenotypic plasticity (PP) and GxEs are hugely important to our understanding of evolution (Via & Lande 1985b; Pigliucci 2009). PP is the expression of different phenotypes in different environments from a single genotype (Whitman & Agrawal 2009). GxEs are when the plastic response (i.e. the change in phenotype with environment) differs among genotypes (Bradshaw 1965; Schlichting & Pigliucci

1998). Their contribution to evolution occurs because PP alters the relationship between an organism's genotype and the phenotypic traits of the organism that are acted on by selection.

In this study, I combined quantitative genetics (QG) and nutritional geometry (NG) to examine how genes (G), the dietary environment (E), and their interaction (GxE), influence a number of important life-history traits (clutch size, diet eaten per day (Diet/Day), gestation period, and offspring lipid proportion (OLP) in female *N. cinerea*. Previous work conducted by myself in a previous study on this species has shown that female *N. cinerea* on a high carbohydrate ('high') diet produce more offspring per clutch and invest more lipids into each offspring than those on a low carbohydrate ('low') diet (Parry et al. 2016). I found that this effect occurred even when females were fed on a 'low' diet for their first clutch, and subsequently shifted to a 'high' diet for their second clutch. This greater investment of lipids permits offspring to survive longer under starvation (Parry et al. 2016). This is important as it suggests that this plastic maternal investment strategy is likely to be adaptive in this species. In this study, I provide clear evidence for ample additive genetic variance (G) and dietary effects (E) in all four traits, as well as significant interactions between the two (GxEs) for Diet/Day and OLP, but not for clutch size or gestation. Our findings demonstrate that complex interactions between genotype and the dietary environment play a critical role in how *N. cinerea* regulate their feeding behaviour and allocate lipid resources into their offspring, specifically how similar genotypes are capable of investing plastically depending on dietary environment. This evidence for PP and GxEs allows us to speculate on the evolutionary trajectory of *N. cinerea* in a fluctuating environment. Plastic maternal investment allows females to increase their fitness through increased offspring survival, maintaining genetic information that would otherwise be lost to selection. By showing that this plasticity is itself based in the genotype of an organism, we can suggest that this PP is impacting the evolution of the species.

Our results show that all traits (clutch size, Diet/Day, gestation, and OLP) show evidence of significant PP across the dietary environments. This is consistent with previous *N. cinerea* studies which have used nutritional geometry (NG) to show that males and females maximise their fitness on high carbohydrate diets (P:C ratio

of approximately 1:4.8 (Bunning et al. 2016)) with increased sex pheromone quantity (Jensen et al. 2015) and quality (Clark et al. 1997), lipid reserves, fast juvenile growth and long reproductive lifespans (Barrett et al. 2009), attractiveness and dominance status (South et al. 2011), and female clutch size and gestation time (Bunning et al. 2016). This is, however, disputed in one study that found male *N. cinerea* have increased sperm production (the major determinant of male fertility) on low carbohydrate diets, and thus regulate their nutrient intake somewhere between both dietary environments to balance the trade-off between pre- (attractiveness) and post- (sperm production) copulatory traits (Bunning et al. 2015). Thus, we chose a 1:8 carbohydrate ratio for this study to maximise female fitness, as males were fed on standard rat chow. More broadly, carbohydrate has been shown to influence offspring traits in a variety of other insect species, including; increased offspring number and size in a polymorphic ant species (*Solenopsis invicta*) (Wills et al. 2015), increased oviposition period, fecundity, total clutch size and total clutch mass in the moth *Ostrinia nubilalis* (Leahy & Andow 1994), and increased investment into sexual traits in the ant *Myrmica brevispinosa* (Bono & Herbers 2003) and the moth *Heliothis virescens* (Willers et al. 1987). For females, we can posit that traits such as gestation time and clutch size are energetically costly traits, supported by the finding that gestation time is negatively correlated with metabolic rate in *N. cinerea* (Schimpf et al. 2013). Despite containing the same energy content gram-for-gram as protein, carbohydrate is an easier source of energy to access metabolically (Cohen 2015), suggesting that females prioritise high carbohydrate diets in order to meet the energy requirements of reproduction. This is supported by the evidence that *N. cinerea*, and most other cockroach species, carry endosymbiotic bacteria (*Blattabacterium*) within specialised cells in the fat body which allow them to store excess nitrogen as uric acid crystals (Kambhampati et al. 2013). This stored nitrogen can be accessed when protein is scarce in the diet, allowing production of amino acids (Sabree et al. 2009; Patiño-Navarrete et al. 2014). Given that the cockroaches in this experiment were reared on rat chow as juveniles (~20% crude protein), it seems likely that they could develop nitrogen stores during the juvenile growth stage that would reduce the need for dietary protein intake in post-eclosion adulthood.

Two of our traits, Diet/Day and OLP, show evidence for significant GxEs. This means that organisms with similar genotypes can alter their behaviour plastically to maximise fitness, and that the plastic response to the novel environment varies between genotypes – a genetic basis for plasticity. In the case of Diet/Day, it appears that organisms on a 'low' diet compensate for the poor nutritional quality of said diet by eating significantly more per day (see reaction norms in Figure 3.1). This evidence of GxEs is important because phenotypic plasticity with a genetic basis that is adaptive (i.e. increases fitness in novel environments) has been shown as fundamentally important for evolution, as without a genetic basis, plasticity cannot evolve (Wund 2012). For Diet/Day, a plastic change in dietary consumption can easily be seen as beneficial for an organism's persistence in a new environment. Should a critical nutrient, such as carbohydrate for *N. cinerea*, become limited, a plastic response to consume more food should individuals to meet their optimum dietary intake of said nutrient, allowing them to survive. Because this plastic response has a genetic basis, the plasticity itself can be passed on to subsequent generations. Similarly, plastic responses in OLP means that females are capable of altering their investment into offspring dependent upon the dietary environment. When in a dietary environment that allows them to invest more into offspring, and increase their fitness, they are capable of doing so. Evidence of plasticity allowing persistence and adaptation to novel environments has been shown in several studies; plasticity in breeding cycles have enabled dark-eyed juncos (*Junco hymenalis*) to successfully colonise new habitats (Yeh & Price 2004), and red squirrels (*Tamiasciurus hudsonicus*) in Canada can cope with climatic change thanks to a genetic basis to plasticity (Réale, McAdam, et al. 2003). These and other studies show evidence that plasticity allows genotypes to persist in new environments for long enough for mutation and recombination to promote evolutionary adaptation to the new environment (Lee & Petersen 2002; Geng et al. 2007).

In contrast to OLP, I did not find any evidence for GxEs for clutch size or gestation. Clutch size of female *N. cinerea* is influenced mostly by genotype. Clutch size had a relatively high heritability (0.434 on 'low', 0.411 on 'high'), and variation in the trait was explained significantly better by a model that included G than one that did not.

This suggests that the clutch size of female *N. cinerea* is strongly linked to the genotype of the organism, rather than the environment in which the organism exists. As expected, individuals in a better nutritional environment (on the 'high' diet), produce significantly larger clutches (see reaction norms in Figure 3.1). This is supported by previous studies on *N. cinerea* that found that clutch size was maximised on a carbohydrate intake of 8 mg per day, and on a protein to carbohydrate ratio of 1:4.8 (Bunning et al. 2016; Parry et al. 2016). Similarly, gestation does not show any evidence of GxEs and is less heritable than clutch size (0.218 on 'low', 0.249 on 'high'). Individuals in a better nutritional environment had significantly shorter gestation periods than those in a nutritionally poor environment (see reaction norms in Figure 3.1), which is also supported by previous studies (Bunning et al. 2016; Parry et al. 2016). Neither of these traits can be said to show a genetic basis for plasticity, rather, their response to environmental factors is limited by their genetic basis.

In conclusion, I can report that female *N. cinerea's* nutritional intake and fitness are both significantly influenced by G, E and GxEs. The data shows that individuals with similar genotypes respond differently to novel environments, exhibiting plasticity. The plastic traits display different levels of heritability, and thus potential for selection and evolution, in each. This ability to adapt phenotypically to an environment without changes to the genome allows individuals to rapidly respond to novel conditions, and persist at least until they are able to procreate. Further, I have ascertained that some of the traits I examined have a base in the individual genome, meaning that the responses themselves can be selected for through the process of evolution. Many have argued that these complex evolutionary outcomes warrant an extension of the Modern Synthesis to a more mechanistic, development-centric viewpoint. This more mechanistic view accounts for the interactions between genetics and the environment in the creation of phenotypes (West-Eberhard 2003; Sultan 2007; Pigliucci & Müller 2010). Our study contributes to a growing portfolio of evidence that plasticity, i.e. the capacity of a single genotype to exhibit a range of phenotypes in response to variation in the environment, a fundamental characteristic of developmental systems, impacts the evolutionary process. Understanding the mechanisms through which organisms

rapidly respond to changing environments is becoming increasingly important as climate change threatens to alter ecosystems throughout our world.

Chapter 4: General Discussion

The link between phenotypic plasticity and the evolutionary process has been explored both theoretically (Via & Lande 1985b; Pigliucci 2007; Pigliucci 2009; Pigliucci 2005; West-Eberhard 2003; Wund 2012; Gibson & Dworkin 2004; Le Rouzic & Carlborg 2008; Schlichting 2008; Baldwin 1896; Morgan 1896; Schmalhausen 1949; Pigliucci 2001; Ghalambor et al. 2007; Lande 2009) and empirically (C.H. Waddington 1952; Badyaev 2009; Ledón-Rettig et al. 2010; Badyaev 2008; Yeh & Price 2004; Réale, McAdam, et al. 2003; Price et al. 2003; Otaki et al. 2010; Nishimura et al. 2010). Through understanding the genetic basis of phenotypic plasticity, we can hope to further our understanding of the evolutionary process and predict how organisms will adapt to changing environments caused by events such as colonisation (Mason 2016; Sidorovich 2014; Mathers et al. 2017; Frenot et al. 1999), and the environmental shifts caused by global climate change (Réale et al. 2003; Charmantier et al. 2008; Merilä & Hendry 2014; Crozier et al. 2008; Seebacher et al. 2014; Chown et al. 2007). While there are many studies searching for the genetic basis of plasticity, exhibited as genotype-by-environment interactions, using a pedigree (Fishback et al. 2002; Dupont-Nivet et al. 2008; Wallenbeck et al. 2009; Nivard et al. 2016; Jannink et al. 2001), there are few that examine it through the lens of nutritional geometry, observing the effects of dietary environment on the plastic expression of complex traits (Bonjour et al. 2007; Reed et al. 2010; Deans et al. 2016).

Recently, there have been an abundance of studies that have used nutritional geometry to better understand the link between complex, quantitative traits and their interaction with the dietary environment (Rapkin et al. 2018; Deans et al. 2015; Bunning et al. 2016; Clark et al. 2014; Fanson & Taylor 2012; House et al. 2016; Jensen et al. 2015; Lee 2015; Maklakov et al. 2008; Roeder & Behmer 2014; Raubenheimer et al. 2014). These studies view the nutritional environment as a landscape of fitness peaks, whereby different fitness-related traits are maximised by consuming specific nutrients in specific ratios, or rails (Simpson et al. 2009; Bunning et al. 2015; Raubenheimer & Jones 2006; South et al. 2011; Simpson & Raubenheimer 1993). My research provides yet more evidence to the growing

body of studies that suggest the intake of two macronutrients, protein and carbohydrate, are fundamentally involved in the expression of quantitative traits that determine, in part, an organism's fitness (see previous references). Relatively few of these studies have used this geometric framework of nutrition to examine phenotypic plasticity however, which my study has attempted to do (Lee et al. 2012; Bonduriansky et al. 2016). By precisely measuring the intake of specific nutrients throughout the adult life of female *N. cinerea*, we have been able to quantify the effects that the nutritional environment has had on an organism's traits, specifically gestation period, clutch size, and offspring lipid proportion.

Previous work I have conducted has shown that *N. cinerea* mothers maximise their reproductive fitness on high carbohydrate diets (Parry et al. 2016). These diets allow mothers to produce significantly more offspring, with significantly larger lipid reserves, that survive significantly longer under food deprivation. Additionally, I showed in that study that when mothers were switched between nutritional environments between clutches, moving onto a high carbohydrate diet allowed them to partially compensate for previously poor nutritional conditions (Parry et al. 2016). I also found that moving on a low carbohydrate diet from a high carbohydrate diet caused the previously mentioned traits to decrease, but that there was some effect of initial diet that prevented the traits falling as low as mothers maintained on low carbohydrate diets for both clutches. This initial demonstration of nutritionally dependent phenotypic plasticity prompted this study, which aims to determine whether said plasticity has a genetic basis.

In addition to my use of nutritional geometry to quantify the effect of nutritional environment on the plasticity of complex traits, I have also created a half-sibling pedigree (in accordance with the outlines defined by Lynch & Walsh 1998, and developed by both Kruuk & Hadfield 2007, and Wilson et al. 2010), to account for the impacts of genotype on the traits, as used in previous studies (Schimpf et al. 2013; Rapkin et al. 2018). While this quantitative genetic approach to examining complex traits has been utilised in a range of fields including developmental biology (Klingenberg & Leamy 2001), sexual conflict (Poissant et al. 2008), metabolism (Reed et al. 2010), and gene mapping (Jannink et al. 2001), its deployment in concurrence with nutritional geometry is rare (Lande 2009). By

combining these two experimental methods, mediated by the use of an animal model (Kruuk 2004; Wilson et al. 2010), I have been able to accurately determine the impact of not only nutritional environment and genotype, but also the genotype-by-environment interaction, on the fitness-related complex traits of female *N. cinerea*.

My research has shown that nutritionally mediated phenotypic plasticity occurs in amount of diet eaten per day, gestation period, clutch size, and offspring lipid proportion of female *N. cinerea*. We have also discovered that there are significant GxE interactions for both amount eaten per day and offspring lipid proportion. This is important because it suggests these traits show plasticity between nutritional environments, and that this plasticity varies among genotypes. Because the response varies among genotypes, selection can act upon this variation, allowing the plastic response itself to evolve (Pigliucci 2005). Given that one of these traits, offspring lipid proportion, is a major determinant of individual fitness (Parry et al. 2016), and is causally impacted by the other trait, amount eaten per day, the implications for the evolution of plasticity in this trait are apparent; an adaptive plastic response to a novel or changing environment will promote adaptive promotes adaptive radiation (Pfennig et al. 2010) and confer transgenerational fitness advantages (Badyaev & Oh 2008). These processes both drastically impact the evolutionary process (Wund 2012).

To summarise, the work in this thesis shows the complex impacts of nutritional environment, genotype, and genotype-by-environment interactions on quantitative traits that impact the fitness of both mother and offspring *N. cinerea*. We have shown that these complex traits vary plastically, and that there is variation within the plastic response among genotypes in the population, providing material on which natural selection can act to cause evolution. The work I have carried out aids our understanding of the evolutionary process, and specifically how organisms can respond to novel and changing environments.

Appendix 1: Exemplar ASReml Code for Animal Models

Text 1. Example ASReml Code for running all models.

```
animal          !P
mate            !P
diet            !A
mum             !A
eaten           !/246.2
prot            !/3.274
carb            !/26.24
gest            !/2.498
off             !*-1 !+41 !^0 !/0.5003
block           !A
eatenday        !/4.546
pday            !/0.6046E-01
cday            !/0.4845
size            !/0.4970
lipmass                 !/54.58
lipprop         !/0.1283
offlipmass      !/0.1500
offlipprop      !^0     !/0.2827
off21           !*-1 !+41 !^0 !/0.5003
off24           !*-1 !+41 !^0 !/0.5003
gest21          !/2.498
gest24          !/2.498
eatenday21      !/4.546
eatenday24      !/4.546
offlipprop21    !^0     !/0.2827
offlipprop24    !^0     !/0.2827

roaches.ped !skip 1

roaches.asd !skip 1 !MVINCLUDE !FCON !DDF   !dopart  7    !maxit 25000     !display 2 #
!continue  #!Filter diet !select 2

###################################

!part 1

off ~ mu diet block          !r animal           #maternal effects bound to zero, good

#eatenday ~ mu diet block    !r animal #mum      # maternal effects marginall NS, but may
need to revisit for publication

#gest ~ mu diet block        !r animal           #maternal effects bound to zero,good

#offlipprop ~ mu diet block  !r animal          #maternal effects bound to zero, good

VPREDICT  !DEFINE
F vp  1+2
H h2  1 3

#####################################

!part 2    # GxE model  for all traits except gestation
```

```
gest21  gest24 ~ Trait  Trait.block     !r Trait.animal

1 2 1
0

Trait 0 US  !S2==1  !GPZP

0.4
0   0.5

Trait.animal  2

Trait 0  US   !GP

0.2343
0.1948      0.1620

animal

VPREDICT  !DEFINE
F VP_21  1+3
F VP_24  2+5
F VA_21   3
F VR_21   1
H h2_21   3 6
F VA_24  5
F VR_24  2
H h2_24  5 7
F COVA    4
R rG      3 4 5

#####################################

!part 3

gest21  gest24  ~ Trait  Trait.block     !r animal

1 2 0
0

Trait 0 US  !S2==1  !GPZP

0.4
0   0.4

#####################################

!part 4

gest21  gest24 ~ Trait  Trait.block     !r Trait.animal

1 2 1
0

Trait 0 US  !S2==1  !GPZP

0.4
0   0.5

Trait.animal  2
```

Trait 0 CORGH

0.999 0.4 0.4

animal

VPREDICT !DEFINE

F VP_21 1+4
F VP_24 2+5
F VA_21   4
F VR_21   1
H h2_21   4 6
F VA_24 5
F VR_24 2
H h2_24 5 7

#####################################

References

Badyaev, A. V., 2009. Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the Baldwin effect. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1520), pp.1125–1141.

Badyaev, A. V., 2008. Maternal effects as generators of evolutionary change: A reassessment. *Annals of the New York Academy of Sciences*, 1133, pp.151–161.

Badyaev, A. V., Foresman, K.R. & Young, R.L., 2005. Evolution of Morphological Integration: Developmental Accommodation of Stress-Induced Variation. *The American Naturalist*, 166(3), pp.382–395.

Badyaev, A. V. & Oh, K.P., 2008. Environmental induction and phenotypic retention of adaptive maternal effects. *BMC Evolutionary Biology*, 8(1), pp.1–10.

Baldwin, J.M., 1896. A New Factor in Evolution. *The American Naturalist*, pp.3354–451.

Barrett, E.L.B. et al., 2009. Separate and combined effects of nutrition during juvenile and sexual development on female life-history trajectories: the thrifty phenotype in a cockroach. *Proceedings of the Royal Society B: Biological Sciences*, 276(June), pp.3257–3264.

Barrett, E.L.B., Moore, A.J. & Moore, P.J., 2009. Diet and social conditions during sexual maturation have unpredictable influences on female life history trade-offs. *Journal of Evolutionary Biology*, 22, pp.571–581.

Berven, K.A., 1982. The Genetic Basis of Altitudinal Variation in the Wood Frod Rana Sylvatica. I. An Experimental Analysis of Life History Traits. *Evolution*, 36(5), pp.962–983.

Bonduriansky, R., Runagall-McNaull, A. & Crean, A.J., 2016. The nutritional geometry of parental effects: maternal and paternal macronutrient consumption and offspring phenotype in a neriid fly. *Functional Ecology*, 30, pp.1675–1686.

Bonjour, J.P. et al., 2007. Gene-environment interactions in the skeletal response to nutrition and exercise during growth. *Medicine and Sport Science*, 51, pp.64–80.

Bono, J.M. & Herbers, J.M., 2003. Proximate and ultimate control of sex ratios in Myrmica brevispinosa colonies. *Proceedings of the Royal Society B: Biological Sciences*, 270(1517), pp.811–817.

Bradshaw, A.D., 1965. Evolutionary Significance of Phenotypic Plasticity in Plants. *Advances in Genetics*, 13(C), pp.115–155.

Bunning, H. et al., 2016. Dietary choice for a balanced nutrient intake increases the mean and reduces the variance in the reproductive performance of male and female cockroaches. *Ecology and Evolution*, 6(14), pp.4711–4730.

Bunning, H. et al., 2015. Protein and carbohydrate intake influence sperm number and fertility in male cockroaches, but not sperm viability. *Proceedings of the Royal Society B: Biological Sciences*, 282, pp.1–8.

C.H. Waddington, 1952. Genetic Assimilation of an Acquired Character. *Evolution*, 7(2), pp.118–126. Available at: http://www.chd.ucsd.edu/_files/winter2009/waddington-assimilation.pdf.

Charmantier, A. et al., 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, 320(5877), pp.800–803.

Chase, J. et al., 1992. Biosynthesis and endocrine control of the production of the German cockroach sex pheromone 3,11-dimethylnonacosan-2-one. *Proceedings of the National Academy of Sciences of the United States of America*, 89(13), pp.6050–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1631090%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC49435.

Chase, J. et al., 1990. Biosynthesis of methyl branched hydrocarbons of the German cockroach Blattella germanica (L.) (orthoptera, blattellidae). *Insect Biochemistry*, 20(2), pp.149–156.

Chown, S.L. et al., 2007. Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proceedings of the Royal Society B: Biological Sciences*, 274(1625), pp.2531–2537.

Clark, D.C., DeBano, S.J. & Moore, A.J., 1997. The influence of environmental quality on sexual selection in Nauphoeta cinerea (Dictyoptera:Blaberidae). *Behavioral Ecology*, 8(1), pp.46–53.

Clark, R.M., Zera, A.J. & Behmer, S.T., 2014. Nutritional physiology of life-history trade-offs: how food protein-carbohydrate content influences life-history traits in the wing-polymorphic cricket Gryllus firmus. *Journal of Experimental Biology*, 218(2), pp.298–308.

Cohen, A.C., 2015. *Insect diets: science and technology*, Boca Raton, Florida, USA: CRC Press.

Crozier, L.G. et al., 2008. Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. *Evolutionary Applications*, 1752(4571), pp.252–270.

Deans, C.A., Sword, G.A. & Behmer, S.T., 2016. Nutrition as a neglected factor in insect herbivore susceptibility to Bt toxins. *Current Opinion in Insect Science*, 15, pp.97–103. Available at: http://dx.doi.org/10.1016/j.cois.2016.04.005.

Deans, C.A., Sword, G.A. & Behmer, S.T., 2015. Revisiting macronutrient regulation in the polyphagous herbivore Helicoverpa zea (Lepidoptera: Noctuidae): New insights via nutritional geometry. *Journal of Insect Physiology*, 81, pp.21–27. Available at: http://dx.doi.org/10.1016/j.jinsphys.2015.06.015.

Dupont-Nivet, M. et al., 2008. Heritabilities and GxE interactions for growth in the European sea bass (Dicentrarchus labrax L.) using a marker-based pedigree. *Aquaculture*, 275(1–4), pp.81–87.

Falconer, D.S., 1960. *Introduction to Quantitative Genetics*, Glasgow.

Falconer, D.S., 1952. The Problem of Environment and Selection. *The American Naturalist*, 86(830), pp.293–298.

Fanson, B.G. & Taylor, P.W., 2012. Protein:carbohydrate ratios explain life span patterns found in Queensland fruit fly on diets varying in yeast:sugar ratios. *Age*, 34(6),

pp.1361–1368.

Fishback, A.G. et al., 2002. Estimates of genetic parameters and genotype by environment interactions for growth traits of rainbow trout (Oncorhynchus mykiss) as inferred using molecular pedigrees. *Aquaculture*, 206(3–4), pp.137–150.

Fisher, R., 1918. The Correlation between Relatives on the Supposition of Mendelian Inheritance. *Transactions of the Royal Society of Edinburgh*, 52, pp.399–433.

Fordyce, J.A., 2006. The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *The Journal of Experimental Biology*, 209(12), pp.2377–2383.

Frenot, Y. et al., 1999. Phenotypic plasticity and genetic diversity in Poa annua L. (Poaceae) at Crozet and Kerguelen Islands (subantarctic). *Polar Biology*, 22(5), pp.302–310.

Geng, Y.P. et al., 2007. Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to colonize a wide range of habitats. *Biological Invasions*, 9(3), pp.245–256.

Ghalambor, C.K. et al., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, 21(3), pp.394–407.

Gibson, G. & Dworkin, I., 2004. Uncovering cryptic genetic variation. *Nature Reviews Genetics*, 5, pp.681–690.

Gilmour, A.R. et al., 2009. ASREML Reference Manual. , pp.1–398. Available at: http://vsni.de/downloads/asreml/release3/UserGuide.pdf%5Cnpapers3://publication/uuid/716D0761-1368-4982-AF2E-F1D8614A80DF.

Harris, W.E. & Moore, P.J., 2005. Female mate preference and sexual conflict: Females prefer males that have had fewer consorts. *The American Naturalist*, 165(May 2005), pp.S64-71.

Harrison, S.J. et al., 2014. Towards a synthesis of frameworks in nutritional ecology:

interacting effects of protein, carbohydrate and phosphorus on field cricket fitness. *Proceedings of the Royal Society B: Biological Sciences*, 281(1792), pp.20140539–20140539.

Hill, W.G., 2010. Understanding and using quantitative genetic variation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1537), pp.73–85.

House, C.M. et al., 2016. Macronutrient balance mediates the growth of sexually selected weapons but not genitalia in male broad-horned beetles. *Functional Ecology*, 30(5), pp.769–779.

Jannink, J.L., Bink, M.C.A.M. & Jansen, R.C., 2001. Using complex plant pedigrees to map valuable genes. *Trends in Plant Science*, 6(8), pp.337–342.

Jensen, K. et al., 2015. Sex-specific effects of protein and carbohydrate intake on reproduction but not lifespan in Drosophila melanogaster. *Aging cell*, 14(4), pp.605–615.

Kambhampati, S., Alleman, A. & Park, Y., 2013. Complete genome sequence of the endosymbiont Blattabacterium from the cockroach Nauphoeta cinerea (Blattodea: Blaberidae). *Genomics*, 102(5–6), pp.479–483. Available at: http://dx.doi.org/10.1016/j.ygeno.2013.09.003.

King, E.G., Roff, D.A. & Fairbairn, D.J., 2011. The Evolutionary Genetics of Acquisition and Allocation in the Wind Dimorphic Cricket, Gryllus firmus. *Evolution*, 65(8), pp.2273–2285.

Klingenberg, C.P. & Leamy, L.J., 2001. Quantitative genetics of geometric shape in the mouse mandible. *Evolution*, 55(11), pp.2342–2352.

Kruuk, L.E.B., 2004. Estimating genetic parameters in natural populations using the "animal model." *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359(1446), pp.873–890.

Kruuk, L.E.B. & Hadfield, J.D., 2007. How to separate genetic and environmental causes of similarity between relatives. *Journal of Evolutionary Biology*, 20(5), pp.1890–1903.

Lande, R., 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology*, 22(7), pp.1435–1446.

Leahy, T.C. & Andow, D.A., 1994. Egg weight, fecundity, and longevity are increased by adult feeding in Ostrinia nubilalis (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America*, 87(3), pp.342–349.

Ledón-Rettig, C.C., Pfennig, D.W. & Crespi, E.J., 2010. Diet and hormonal manipulation reveal cryptic genetic variation: Implications for the evolution of novel feeding strategies. *Proceedings of the Royal Society B: Biological Sciences*, 277(1700), pp.3569–3578.

Lee, C.E. & Petersen, C., 2002. Genotype-by-Environment Interaction for Salinity Tolerance in the Freshwater-Invading Copepod Eurytemora affinis. *Physiological and Biochemical Zoology*, 75(4), pp.335–344.

Lee, K.P., 2015. Dietary protein: Carbohydrate balance is a critical modulator of lifespan and reproduction in Drosophila melanogaster: A test using a chemically defined diet. *Journal of Insect Physiology*, 75(February), pp.12–19. Available at: http://dx.doi.org/10.1016/j.jinsphys.2015.02.007.

Lee, K.P., Kwon, S.T. & Roh, C., 2012. Caterpillars use developmental plasticity and diet choice to overcome the early life experience of nutritional imbalance. *Animal Behaviour*, 84(4), pp.785–793. Available at: http://dx.doi.org/10.1016/j.anbehav.2012.06.033.

Lynch, M. & Walsh, B., 1998. *Genetics and Analysis of Quantitative Traits*, Oxford: Sinauer Associates.

Maklakov, A.A. et al., 2008. Sex-Specific Fitness Effects of Nutrient Intake on Reproduction and Lifespan. *Current Biology*, 18(14), pp.1062–1066.

Martin, M.M., Jones, C.G. & Bernays, E.A., 1991. The Evolution of Cellulose Digestion in Insects [and Discussion]. *Proceedings of the Royal Society B: Biological Sciences*, 333, pp.281–288.

Mason, P.A., 2016. On the role of host phenotypic plasticity in host shifting by parasites. *Ecology Letters*, 19(2), pp.121–132.

Mathers, T.C. et al., 2017. Rapid transcriptional plasticity of duplicated gene clusters enables a clonally reproducing aphid to colonise diverse plant species. *Genome Biology*, 18(27), pp.1–20.

Merilä, J. & Hendry, A.P., 2014. Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, 7(1), pp.1–14.

Merilä, J., Kruuk, L.E.B. & Sheldon, B.C., 2001. Cryptic evolution in a wild bird population. *Nature*, 412(July), pp.1999–2002.

Milner, J.M. et al., 2000. Estimating variance components and heritabilities in the wild: a case study using the “animal model” approach. *Journal of Evolutionary Biology*, 13, pp.804–813.

Monaghan, P., 2008. Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society of London B*, 363(November 2007), pp.1635–1645.

Moreno, J. et al., 2008. Pre-laying nutrition mediates maternal effects on offspring immune capacity and growth in the pied flycatcher. *Oecologia*, 156(4), pp.727–735.

Morgan, C.L., 1896. On Modification and Variation. *Science*, 4(99), pp.733–740.

Mousseau, T. a & Fox, C.W., 1998. The adaptive significance of maternal effects. *Trends in ecology & evolution*, 13(10), pp.403–407.

Nestel, D. et al., 2016. Resource allocation and compensation during development in holometabolous insects. *Journal of Insect Physiology*, 95, pp.78–88. Available at: http://dx.doi.org/10.1016/j.jinsphys.2016.09.010.

Nishimura, N. et al., 2010. Cryptic Genetic Variation and Body Size Evolution in Threespine Stickleback. *Evolution*, 65(4), pp.1203–1211.

Nivard, M.G. et al., 2016. Detection of gene-environment interaction in pedigree data

using genome-wide genotypes. *European Journal of Human Genetics*, 24(12), pp.1803–1809. Available at: http://dx.doi.org/10.1038/ejhg.2016.88.

Nussey, D.H. et al., 2012. Selection on Heritable Phenotypic Plasticity in a Wild Bird Population. *Science*, 310, pp.304–306.

Otaki, J.M. et al., 2010. Phenotypic plasticity in the range-margin population of the lycaenid butterfly Zizeeria maha. *BMC Evolutionary Biology*, 10(1).

Parry et al., 2016. Undergraduate thesis: Diet Dependent Plasticity in the Cockroach *Nauphoeta cinerea. Unpublished findings.*

Patiño-Navarrete, R. et al., 2014. The cockroach Blattella germanica obtains nitrogen from uric acid through a metabolic pathway shared with its bacterial endosymbiont. *Biology Letters*, 10(7), pp.20140407–20140407.

Pemberton, J.M., 2008. Wild pedigrees: the way forward. *Proceedings of the Royal Society B: Biological Sciences*, 275(March).

Pfennig, D.W. et al., 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology and Evolution*, 25(8), pp.459–467. Available at: http://dx.doi.org/10.1016/j.tree.2010.05.006.

Pigliucci, M., 2009. An Extended Synthesis for Evolutionary Biology. *Annals of the New York Academy of Sciences*, 1168(1), pp.218–228. Available at: http://doi.wiley.com/10.1111/j.1749-6632.2009.04578.x.

Pigliucci, M., 2007. Do we need an extended evolutionary synthesis? *Evolution*, 61(12), pp.2743–2749.

Pigliucci, M., 2005. Evolution of phenotypic plasticity : where are we going now ? *Trends in Ecology & Evolution*, 20(9), pp.481–486.

Pigliucci, M., 2001. *Phenotypic Plasticity: Beyond Nature and Nurture (Synthesis in Ecology and Evolution)*, Baltimore: John Hopkins University Press.

Pigliucci, M. & Müller, G.B., 2010. Elements of an Extended Evolutionary Synthesis.

Elements of an extended evolutionary synthesis, (March), pp.3–18.

Poissant, J. et al., 2008. Quantitative genetics and sex-specific selection on sexually dimorphic traits in bighorn sheep. *Proceedings of the Royal Society B: Biological Sciences*, 275(1635), pp.623–628.

Postma, E. & Noordwijk, A.J. Van, 2005. Gene flow maintains a large genetic difference in clutch size at a small spatial scale. *Nature*, 385(1981), pp.65–68.

Price, T.D., Qvarnström, A. & Irwin, D.E., 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences*, 270(1523), pp.1433–1440.

Rapkin, J. et al., 2018. Genotype-by-sex-by-diet interactions for nutritional preference, dietary consumption, and lipid deposition in a field cricket. *Heredity*, 121, pp.361–373. Available at: http://dx.doi.org/10.1038/s41437-018-0130-x.

Raubenheimer, D. et al., 2014. Nutritional Geometry: From Insects to Ruminants. *Proceedings of the Australian Society of Animal Production*, 30(November), pp.32–36.

Raubenheimer, D. & Jones, S.A., 2006. Nutritional imbalance in an extreme generalist omnivore: tolerance and recovery through complementary food selection. *Animal Behaviour*, 71(6), pp.1253–1262.

Réale, D., McAdam, A.G., et al., 2003. Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society B: Biological Sciences*, 270(1515), pp.591–596.

Réale, D., Berteaux, D., et al., 2003. Lifetime Selection on Heritable Life-History Traits in a Natural Population of Red Squirrels. *Evolution*, 57(10), pp.2416–2423.

Reed, L.K. et al., 2010. Genotype-by-diet interactions drive metabolic phenotype variation in Drosophila melanogaster. *Genetics*, 185(3), pp.1009–1019.

Roeder, K.A. & Behmer, S.T., 2014. Lifetime consequences of food protein-carbohydrate content for an insect herbivore. *Functional Ecology*, 28(5), pp.1135–1143.

Le Rouzic, A. & Carlborg, Ö., 2008. Evolutionary potential of hidden genetic variation. *Trends in Ecology and Evolution*, 23(1), pp.33–37.

Sabree, Z.L., Kambhampati, S. & Moran, N.A., 2009. Nitrogen recycling and nutritional provisioning by Blattabacterium, the cockroach endosymbiont. *Proceedings of the National Academy of Sciences*, 106(46), pp.19521–19526.

Schal, C. et al., 1991. Biochemistry and Regulation of Pheromone Production in Blattella Germanica (L.) (Dictyoptera Blatellidae). *Insect Biochemistry*, 21(1), pp.73–79.

Schimpf, N.G., Matthews, P.G.D. & White, C.R., 2013. Discontinuous gas exchange exhibition is a heritable trait in speckled cockroaches Nauphoeta cinerea. *Journal of Evolutionary Biology*, 26, pp.1588–1597.

Schlichting, C.D., 2008. Hidden Reaction Norms, Cryptic Genetic Variation, and Evolvability. *Annals of the New York Academy of Sciences*.

Schlichting, C.D. & Pigliucci, M., 1998. *Phenotypic Evolution: A Reaction Norm Perspective.*, Sunderland: Sinauer Associates Incorporated.

Schmalhausen, I.I., 1949. *Factors of Evolution: The Theory of Stabilizing Selection*, Blakiston, Philadelphia, PA.

Seebacher, F., White, C.R. & Franklin, C.E., 2014. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature*, 5(1), pp.61–66.

Sidorovich, V.E., 2014. Reproductive plasticity of the American mink Mustela vison in Belarus. *Acta Theriologica*, 38(2), pp.175–183.

Simpson, S.J. et al., 2009. Modelling nutritional interactions: from individuals to communities. *Trends in Ecology and Evolution*, 25(1), pp.53–60.

Simpson, S.J. & Abisgold, J.D., 1985. Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. *Physiological Entomology*, 10(4), pp.443–452. Available at: http://doi.wiley.com/10.1111/j.1365-3032.1985.tb00066.x.

Simpson, S.J. & Raubenheimer, D., 1993. A Multi-Level Analysis of Feeding Behaviour: The

Geometry of Nutritional Decisions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 342(1302), pp.381–402.

Simpson, S.J. & Raubenheimer, D., 1995. The Geometric Analysis of Feeding and Nutrition: a User's Guide. *Journal of Insect Physiology*, 41(7), pp.545–553.

Simpson, S.J. & Raubenheimer, D., 2012. *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*, Princeton, New Jersey: Princeton University Press.

Solemdal, P., 1997. Maternal effects - A link between the past and the future. *Journal of Sea Research*, 37(3–4), pp.213–227.

Sorensen, D. & Gianola, D., 2002. *Likelihood, Bayesian, and MCMC Methods in Quantitative Genetics*, New York: Springer.

South, S.H. et al., 2011. Male cockroaches prefer a high carbohydrate diet that makes them more attractive to females: Implications for the study of condition dependece. *Evolution*, pp.1594–1606.

Sultan, S.E., 2007. Development in context: the timely emergence of eco-devo. *Trends in Ecology and Evolution*, 22(11), pp.575–582.

Trivers, R. L. & Willard, D. E. 1973 Natural selection of parental ability of vary the sex ratio of offspring. *Science* 179, 90–92. (doi:10.1126/science.179.4068.90)

Uller, T., 2008. Developmental plasticity and the evolution of parental effects. *Trends in Ecology & Evolution*, 23(8), pp.432–438. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0169534708001894.

Via, S., 1984. The Quantitative Genetics of Polyphagy in an Insect Herbivore. I. Genotype - Environment Interaction in Larval Performance on Different Host Plant Species. *Evolution*, 38(4), pp.881–895.

Via, S. & Lande, R., 1985a. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*, 39(3), pp.505–522.

Via, S. & Lande, R., 1985b. Genotype-Environment Interaction and the Evolution of Phenotypic Plasticity. *Evolution*, 39(3), pp.505–522.

Wallenbeck, A., Rydhmer, L. & Lundeheim, N., 2009. GxE interactions for growth and carcass leanness: Re-ranking of boars in organic and conventional pig production. *Livestock Science*, 123(2–3), pp.154–160. Available at: http://dx.doi.org/10.1016/j.livsci.2008.11.003.

Warner, D. a, Lovern, M.B. & Shine, R., 2007. Maternal nutrition affects reproductive output and sex allocation in a lizard with environmental sex determination. *Proceedings. Biological sciences / The Royal Society*, 274(1611), pp.883–890.

West-Eberhard, M., 2005. Developmental plasticity and the origin of species differences. *… of the National Academy of Sciences*, 102, pp.6543–6549. Available at: http://www.pnas.org/content/102/suppl_1/6543.short.

West-Eberhard, M.J., 2003. *Developmental Plasticity and Evolution.*, Oxford University Press.

West-Eberhard, M.J., 2003. *Developmental Plasticity and Evolution*, Oxford: Oxford University Press.

Whitman, D.W. & Agrawal, A.A., 2009. *What is Phenotypic Plasticity and Why is it Important?* 1st ed., New Hampshire: Science Publishers.

Willers, J.L., Schneider, J.C. & Ramaswamy, S.B., 1987. Fecundity, longevity and caloric patterns in female Heliothis virescens: Changes with age due to flight and supplemental carbohydrate. *Journal of Insect Physiology*, 33(11), pp.803–808.

Wills, B.D. et al., 2015. Effect of Carbohydrate Supplementation on Investment into Offspring Number, Size, and Condition in a Social Insect. *Plos One*, 10(7), p.e0132440.

Wilson, A.J. et al., 2010. An ecologist's guide to the animal model. *Journal of Animal Ecology*, 79, pp.13–26.

Wolf, J.B. & Wade, M.J., 2016. The evolutionary genetics of maternal effects. *Evolution*. Available at:

http://books.google.com/books?hl=en&lr=&id=JuARTAPwNzUC&oi=fnd&pg=PA5&dq=The+evolutionary+genetics+of+maternal+effects&ots=n7DDwbTnfM&sig=wG6UFr_dcUC_bA4rc5TuiOtVazs.

Wolf, J.B. & Wade, M.J., 2009. What are maternal effects (and what are they not)? , (March), pp.1107–1115.

Wright, S., 1931. Evolution in Mendelian Populations. *Genetics*, 16, pp.97–159. Available at: https://scholar.google.co.id/citations?view_op=view_citation&continue=/scholar%3Fhl%3Den%26as_sdt%3D0,5%26scilib%3D1&citilm=1&citation_for_view=_9294oEAAAAJ:eflP2zaiRacC&hl=en&oi=p.

Wund, M.A., 2012. Integrative and Comparative Biology Assessing the Impacts of Phenotypic Plasticity on Evolution. *Integrative and Comparative Biology*, 52(1), pp.5–15.

Yeh, P.J. & Price, T.D., 2004. Adaptive Phenotypic Plasticity and the Successful Colonization of a Novel Environment. *The American Naturalist*, 164(4), pp.531–542.